OIPE JC145
MAY 2 5 2005
PATENT & TRADEMARK OFFICE

DOCKET NO.: ISPH-0595US.P1 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 10/684,440 Confirmation No.: 4998

Applicant : Crooke et al.

Filed: : October 15, 2003

TC/A.U. : 1635

Examiner : A. Bowman

Customer No. : 36441

Title : MODULATION OF APOLIPOPROTEIN (A)

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 CFR § 1.132

Sir:

I, Mark J. Graham, residing at 2305 S. Ola Vista, San Clemente, California, 92672, a citizen of the United States of America, do declare and state that:

1. I am one of the named joint inventors of the subject matter claimed in the above-identified patent application.

2. This Declaration is submitted in the above-identified application in response to the Examiner's rejection under 35 USC § 112, first paragraph in the Office Action dated January 26, 2004.

3. This Declaration presents additional evidence that supports the original disclosure in the specification regarding *in vivo* efficacy of apolipoprotein(a) antisense compounds described in the specification. Particularly, this evidence is presented in further support of the presently pending claims to an *in vivo* method of inhibiting expression of apolipoprotein(a) in cells or tissues by contacting cells or tissues with a compound of the present invention.

This data is conducted using an animal model, i.e., a murine model. The test compound is 15 to 30 nucleobases in length targeted to a nucleic acid molecule encoding murine apolipoprotein(a), wherein the compound specifically hybridizes with the nucleic acid molecule encoding Apolipoprotein(a) and inhibits the expression of Apolipoprotein(a). As taught by the specification, the method is useful in *in vitro* testing of cells and in *in vivo* use in humans and animals to inhibit apolipoprotein(a) expression.

4. The following experiment provides support for the *in vivo* method, and was performed by me or under my direction and control.

5. In accordance with the present invention, a compound of the invention, i.e., ISIS 144379 (SEQ ID NO: 23) was evaluated in a dose-response study in mice transgenic for both human apolipoprotein(a) and human apolipoprotein B, and in mice transgenic for human apolipoprotein B alone. ISIS 144379 (acctgacaccgggatccctc) is a chimeric oligonucleotide ("gapmer") 20 nucleotides in length, composed of a

central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines.

6. Eight mice were divided into two groups; (1) control (n=4) and (2) ISIS 144379-injected animals (25 mg/kg; n=4). Animals in each group received either a saline injection or an antisense oligonucleotide injection with ISIS 144379 at 25 mg/kg two times per week. All injections were administered intraperitoneally.

Following sacrifice of the mice after a four-week investigation period, liver and blood samples were collected. Control animals were evaluated for apolipoprotein(a) mRNA levels by real-time PCR. Data represent the average of results from four animals per treatment and are presented in the following Table A.

TABLE A

Effects of Antisense Compounds on Apolipoprotein(a)

	mRNA expression % control	
Transgene	**apoB**	**apo(a)**
apolipoprotein B	101	0
apolipoprotein B apolipoprotein(a)	133	61

7. These data demonstrate that reduction of apolipoprotein(a)can be achieved *in vivo* in a mouse model by administration of the antisense oligonucleotides of

the present invention. This mouse model is an accepted analog of human response.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5/24/05

By: Mark J. Graham

OIPE JC145
MAY 25 2005
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Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 CFR § 1.131

Sir:

We, Mark J. Graham, residing at 2305 S. Ola Vista, San Clemente, California, 92672, a citizen of the United States of America, and Rosanne M. Crooke, residing at 3211 Piragua Street, Carlsbad, California, 92009, a citizen of the United States of America, do declare and state that:

1. We are the named co-inventor(s) of the above-identified patent application.

2. This Declaration is submitted to establish conception of the invention in this application in the United States, a NAFTA country after December 8, 1993, or a WTO member country after January 1, 1996 at a date

prior to the effective date of January 15, 2001 (herein after "the Effective Date"), coupled with due diligence in reduction to practice in the United States, a NAFTA country after December 8, 1993, or a WTO member country after January 1, 1996, from prior to said effective date to subsequent reduction to practice. The evidence in the attached computer-generated oligonucleotide synthesis history pages supports conception prior to January 15, 2001 through to a reduction to practice. The reduction to practice is supported by the filing of the United States Patent Application No. 09/923,515, filed August 7, 2001 (hereafter "parent application"), from which the present continuation-in-part United States Patent Application No. 10/684,440 claims priority.

3. The Effective Date of January 15, 2001 identified in the previous paragraph is the date prior to the date the publisher is believed to have mailed the document Frank, S. *et al.* "Adenovirus-mediated apo(a)-antisense-RNA expression efficiently inhibits apo(a) synthesis *in vitro* and *in vivo*," Gene Therapy, 8: 425-430 (2001) (hereinafter "Frank") to subscribers or otherwise published the document. The publisher did not have the actual mail date available, but as noted upon the document, the document was only accepted for publication on January 15, 2001. Therefore, it would not have published prior to that date. Frank was cited by the Examiner in the Office Action dated January 26, 2005 in this application, when combined with other references under 35 USC §103(a) as a basis for an obviousness rejection of claims 2-18, 22 and 25.

4. To establish conception of the invention of this application, computer-generated documents are provided which provide evidence that oligonucleotide sequences which comply with all limitations of the above-noted claims were synthesized by the co-inventors or under their direction prior to the Effective Date. These pages labeled Exhibit A, pages 1-4, are provided and submitted as evidence. The synthesis of these oligonucleotides was conducted by us or under our supervision. These exhibits represent experiments performed in the United States, a NAFTA country after December 8, 1993, or a WTO member country after January 1, 1996, prior to the Effective Date. All dates on these notebook pages have been masked.

5. Exhibit A is a computer-generated record of Order Information for oligonucleotide synthesis of an order identified by ID No. 102817. The relevant columns on this document are the leftmost column 1, which identifies the oligonucleotide sequence by ISIS number; column 4, which provides the identity of the person who ordered the synthesis, i.e., Mark Graham; column 5 which indicates the date of the order (masked); columns 14 and 15, which indicate the date synthesis was started and completed, respectively (masked). Columns 8, 16 and 17 are all marked with the code "S", which is identified on page 4 as representing "SYNTHESIS". All of the dates, which are masked on this document, occurred prior to the Effective Date.

6. The sequences of the oligonucleotides are represented by the ISIS Numbers ranging from 144357 to

144401. An ISIS Number is applied to a specific sequence at the time the sequence is identified and used to refer to that sequence thereafter. The ISIS numbers on Exhibit A thus refer to the same sequences identified in the above-identified patent application and correlate with the same ISIS Number as appear on page 82, Table 1 of the above-identified patent application and on page 85, Table 1 of the parent application, filed August 7, 2001.

7. The undersigned co-inventors do declare and say that Mark Graham ordered the synthesis of oligonucleotides represented by the indicated ISIS numbers prior to the Effective Date; that the data on Exhibit A is accurate; that the correspondence of ISIS number and oligonucleotide sequence is the same on Exhibit A as it is represented in the pending patent application and parent patent applications identified herein; and that all dates of synthesis request and completion reported on Exhibit A occurred prior to the Effective Date.

8. We, the inventors, worked continuously from the date we conceived the invention and performed the acts reported in Exhibit A to at least the time of the filing of the parent application, namely August 7, 2001 and further to the filing date of the present application, namely, October 15, 2003, by performing additional experiments on this invention and assisting in the drafting of this patent document. To establish diligence to reduction to practice of the invention of the present and parent applications, the computer generated order history pages labeled Exhibit A are provided and

submitted as evidence. These exhibits represent experiments performed in the United States, a NAFTA country after December 8, 1993, or a WTO member country after January 1, 1996 from conception up to the filing of the present and parent applications. All dates on these pages of Exhibit A have been masked.

9. Our invention occurred prior to the Effective Date of January 15, 2001, and thus before the publication of Frank.

10. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5/24/05 By:
Mark J. Graham, Ph.D.

Date: 5/24/05 By: Rosanne M Crooke
Rosanne M. Crooke, Ph.D.

Exhibit A

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ISIS PHARMACEUTICALS

RESEARCH INFORMATICS

HOW TO... | BIRD QUICK QUERIES | SEQUENCE SEARCHES | APPLICATIONS | DOCUMENTATION | LINKS & TOOLS | CONTACT US | HELP

Order Information for OrderID 102817

● Complete Order History ○ Current Order Info

ISIS Number	Lot #	OrderID	Ordered By	Order Date	Amount Ordered	Syn #	Syn Status	Template #	Type	Barcode	Well	Synthesis Location	Date Synthesis Started	Date Synthesis Completed	Lot Status	Oligo Order Statu
144367	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	E02	IDT			S	S
144368	3	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	D02	IDT			S	S
144369	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	C02	IDT			S	S
144370	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	B05	IDT			S	S
144371	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	H04	IDT			S	S
144372	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	G04	IDT			S	S
144373	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	F04	IDT			S	S
144374	1	102817	MGRAHAM		0.10 (UMOL)	1	S	9857	Plate	BN2061	E04	IDT			S	S

144375	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	D04	IDT	S	S
144376	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	C04	IDT	S	S
144377	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	B04	IDT	S	S
144378	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	A04	IDT	S	S
144379	2	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	H03	IDT	S	S
144380	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	G03	IDT	S	S
144381	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	F03	IDT	S	S
144382	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	E03	IDT	S	S
144383	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	D03	IDT	S	S
144384	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	C03	IDT	S	S
144385	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	B03	IDT	S	S
144386	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	A03	IDT	S	S
				0.10									

144387	1	102817	MGRAHAM	(UMOL)	1	S	9857	Plate	BN2061	H02	IDT	S	S
144388	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	G02	IDT	S	S
144389	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	F02	IDT	S	S
144390	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	B02	IDT	S	S
144391	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	A02	IDT	S	S
144392	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	H01	IDT	S	S
144393	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	G01	IDT	S	S
144394	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	F01	IDT	S	S
144395	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	E01	IDT	S	S
144396	2	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	D01	IDT	S	S
144397	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	C01	IDT	S	S
144398	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	B01	IDT	S	S
144399	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	A01	IDT	S	S

144400	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	C05	IDT	S	S
144401	1	102817	MGRAHAM	0.10 (UMOL)	1	S	9857	Plate	BN2061	A05	IDT	S	S

Status Code Key

Status Code	Description
AV	Available
C	Cancelled
CBIS	Imported from CBIS database
D	Delivered
F	Fail
P	Pass
Q ; Plate_Queue	Queued for synthesis
R	Requeued
S	Synthesis
U	Undetermined